AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A computer implemented method of localizing a biomarker within a cell, comprising:

identifying portions of a first image of the cell that corresponds to a first defined area;

identifying portions of a second image of the cell that corresponds to at least one biomarker; and

superimposing portions of the second image against portions of the first image to identify whether the biomarker is localized within the defined area.

2. (Original) The method of claim 1, wherein the portions of the first image and portions of the second image comprise pixels.

3. (Previously presented) The method of claim 1, wherein the portions of the first image comprise pixels having an intensity corresponding to the first defined area of the cell.

4. (Previously presented) The method of claim 3, further comprising determining the intensity corresponding to the first defined area of the cell.

5. (Previously presented) The method of claim 2, wherein the portions of the second image comprise pixels having an intensity corresponding to at least one biomarker.

6. (Previously presented) The method of claim 5, further comprising determining the intensity corresponding to at least one biomarker.

7. (Original) The method of claim 1, further comprising reducing representation of out-of-focus elements in the first image.

8. (Original) The method of claim 7, wherein reducing representation comprises manipulating image pixel intensities of the first image based on image pixel intensities of a third image featuring a different depth of focus.

9. (Original) The method of claim 1, wherein the defined area is selected from the group consisting of the cell nucleus, cytoplasm, nuclear membrane, cellular membrane, mitochondria, endoplasmic reticulum, peroxisome and lysosome.

10. (Previously presented) The method of claim 1, wherein the biomarker is selected from the group consisting of a protein, peptide, nucleic acid, lipid or carbohydrate.

11. (Previously presented) A method of localizing a biomarker within a sub-cellular compartment of a cell, comprising:

obtaining a first image of a cell treated with a first stain that is selective for a first sub-cellular compartment within the cell and a second stain that is selective for at least one biomarker;

determining an intensity value for the first stain at a plurality of pixel locations in the first image;

based on the intensity values, determining pixel locations in the first image that correspond to the first sub-cellular compartment within the cell and assigning those pixel locations to the first sub-cellular compartment;

obtaining a second image of the cell and determining an intensity value for the second stain at a plurality of pixel locations in the second image; and

comparing the first and second images, wherein the presence of pixel locations in the second image that are within the first sub-cellular compartment indicate that the biomarker is within the first sub-cellular compartment.

12. (Previously presented) The method of claim 11, wherein the first sub-cellular compartment is selected from the group consisting of the cell nucleus, cytoplasm, nuclear membrane, cellular membrane, mitochondria, endoplasmic reticulum, peroxisome and lysosome.

13. (Previously presented) The method of claim 11, wherein the biomarker is selected from the group consisting of a protein, peptide, nucleic acid, lipid or carbohydrate.

14. (Original) The method of claim 11, wherein the cell is contacted with a third stain that is selective for a second defined area within the cell, the method further comprising:

in a third image of the distribution of the third stain in the cell, determining an intensity value for each pixel location of a plurality of pixel locations in the third image;

based on the intensity values, determining which pixel locations in the third image correspond to the second defined area of the cell and assigning those pixel locations to the second defined area; and

identifying which of the pixel locations in the second image are within the second defined area.

15. (Original) The method of claim 14, further comprising assigning pixel locations not assigned to the first or second defined areas to a third defined area, and identifying which of the pixel locations in the second image are within the third defined area.

16. (Original) The method of claim 15, wherein the first, second, and third defined areas are selected from the group consisting of: a nucleus, cytoplasm, nuclear membrane, cellular membrane, mitochondria, endoplasmic reticulum, peroxisome and lysosome.

17. (Previously presented) The method of claim 14, wherein the cell is contacted with a fourth stain that is selective for a defined area in the cell, and at least one pixelated image of the distribution of the fourth stain is acquired, the method further comprising reading a third intensity value for each of a plurality of pixels in the image of the fourth stain distribution;

determining a threshold intensity value from the third intensity values;

comparing the third intensity value for each of the plurality of pixels to the threshold intensity; and

assigning pixel locations to a mask based on the threshold intensity value.

18. (Currently Amended) The method of claim 17, wherein the pixel locations in the plurality of pixels in the image of the first stain distribution are the pixel locations in the mask [set].

19. (Original) The method of claim 18, wherein the pixel locations assigned to the mask comprise the location of pixels having third intensity values equal to or greater than the threshold intensity value.

20. (Original) The method of claim 19, further comprising binning the third intensity values for each of the plurality of pixels in the image of the fourth stain distribution.

21. (Currently Amended) The method of claim 20, wherein the threshold intensity value is determined from an intensity value of a largest bin, where the largest bin is the most common pixel intensity.

22. (Currently Amended) The method of claim 20, wherein the threshold intensity value is determined from an intensity value of a second largest bin, where the second largest bin is a lower pixel intensity than the largest bin.

23. (Original) The method of claim 17, further comprising comparing for each pixel location the first intensity value to the second intensity value and assigning the pixel location to the second defined area when the second intensity value is greater than the first intensity value.

24. (Original) The method of claim 17, further comprising reading a signal intensity value for each pixel location in an array of pixels in the image of the second stain distribution, and summing the signal intensity values to determine a total signal intensity.

25. (Original) The method of claim 17, wherein the array of pixels in the image of the second stain distribution is the first defined area in the cell.

26. (Original) The method of claim 17, further comprising reading a signal intensity value for each pixel location in an array of pixels in the image of the second stain distribution, and summing the signal intensity values.

27. (Original) The method of claim 17, wherein the array of pixels in the image of the second stain distribution is the second defined area.

28. (Original) The method of claim 17, further comprising reading a signal intensity value for each pixel location in an array of pixels in the image of the second stain distribution, and summing the signal intensity values to determine a total intensity.

29. (Original) The method of claim 17, wherein the array of pixels in the image of the second stain distribution is the third defined area.

30. (Previously presented) A method of localizing a biomarker within a sub-cellular compartment comprising:

accessing a value for each of a plurality of pixel locations of a stained sub-cellular compartment obtained in a first image of a cell;

accessing a value for each of the plurality of pixel locations of a stained biomarker obtained in a second image of a cell; and

subtracting a percentage of the intensity value for each pixel location in the second image from the intensity value of the same pixel location in the first image to obtain an adjusted intensity value, indicative of the biomarker within the sub-cellular compartment.

31. (Original) The method of claim 30, wherein the first image is acquired at a first focal plane and the second image is acquired at a second focal plane.

32. (Original) The method of claim 30, wherein the percentage of the intensity value for each pixel location in the second image subtracted is determined from an intensity distribution function.

33. (Withdrawn) A method of analyzing a plurality of spots, comprising:

locating the spots;

defining a reference point for the located spot;

connecting the reference point of the located spot to the reference point of a set of nearest neighbor spots with a corresponding line segment; and

identifying points of intersection between line segments used to connect spots to each other.

34. (Withdrawn) The method of claim 33, further comprising assigning reference points and points of intersection a reference number.

35. (Withdrawn) The method of claim 34, further comprising tabulating the reference numbers and spot locations.

36. (Withdrawn) The method of claim 33, wherein the reference point within each spot is the center of the spot.

37. (Withdrawn) The method of claim 33, wherein the spots are histospots in a tissue microarray.

38. (Withdrawn) The method of claim 33, further comprising connecting each of a plurality of reference points to a nearest edge using a corresponding line segment.

39. (Previously presented) The method of claim 11, wherein the stain is a fluorophore.

40. (Previously presented) The method of claim 11, wherein the cell has been fixed.

41. (Previously presented) The method of claim 30, wherein the cell has been fixed.

42. (Previously presented) The method of claim 11, wherein the cell is in a tissue.

43. (Previously presented) The method of claim 30, wherein the cell is in a tissue.